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Original Research

Analysis of genetic sequence variation in *Formicidae* NCBI popset 1871743971 using in silico RFLP

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Abstract

In practically every region of the world, with the exception of the poles, ants are a common form of insect. This one organism lives in colonies and cooperates, therefore these insects are referred to as social insects. In an ant colony, three types of castes have their respective roles and functions, namely, there are queen ants, worker ants, and male ants. In one ant species, many colonies live scattered and each of them has different genes that are influenced by environmental factors and other factors. The purpose of this study was to determine and analyze the genetic variation of ants with the WNT-1 gene sequence in NCBI popset 1871743971. This research was conducted in May-June 2023 using the RFLP method *in silico.* Based on the results of the study, it was found that there were genetic variations in the WNT-1 gene sequence in *Formicidae* available at NCBI in PopSet 1871743971.

Introduction

Ants (Hymenoptera: Formicidae) are insects that can be found in almost all parts of the world except the polar regions. Ants are the most common group of terrestrial animals found in the tropics. Of the total 750,000 species of insects in the world, 9,500 or 1.27% of them are ants (Latumahina et al., 2013) and can form 15 - 25% of terrestrial animal biomass (Abdul-Rassoul et al., 2013). Ants have many functions, one of which is the ecological function of helping plants spread seeds (dispersal), loosening the soil, as predators or predators of other insects (Mele et al.; Orivel & Leroy, 2010), and helping control agricultural pests (Mele et al.; Orivel & Leroy, 2010 and Van Mele & Cuc, 2000).

This species comes from the order Hymenoptera, family Formicidae and is a type of insect that is easily found in abundance in forest ecosystems. Ants have various uses, namely as predators, detrivores, decomposers, decomposers and bioindicators of forest ecosystem health (Wilkie et al. 2010). But unfortunately, the existence of ants in the last decade is thought to have undergone a very large change in composition due to human pressure and pressure from nature. The presence of humans through logging activities, clearing fields, gardens, settlements, physical development, and burning forests greatly influences the composition and distribution of ants in nature. (Fransina Latumahina et al. 2015).

Genetic variation is variation that occurs in the genome of an organism either in nucleotide bases, genes or chromosomes. Genetic variation at the basic level is shown by differences in the nucleotide base sequences (adenine, thymine, guanine and cytosine) that make up DNA in cells

(Harrison, Laverty, and Sterling, 2004). The origins of genetic variation include migration, mutation and recombination (Griffiths et al., 2000). If this genetic variation occurs in a population, it will affect the survival ability of an individual (Frankham et al., 2002). The higher the genetic variation in a population, the better the ability of individuals to adapt to environmental changes (Dunham, 2004).

Genetic diversity at the population, individual and species level can be analyzed based on different marker profiles, both molecular and protein. Markers or markers commonly used in discussing genetic diversity include Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Random Fragment Length Polymorphism (RFLP), Single Sequence Repeats (SSRs) as well as isozymes and allozymes. Random Fragment Length Polymorphism (RFLP) analysis is a technique commonly used to detect genetic variation at the DNA sequence level (Zulfahmi, 2013).

WNT-1 gene Also known as wg wingless in *Drosophila melanogaster* (fruit fly). Enables several functions, including extracellular matrix binding activity; heparan sulfate proteoglycan binding activity; and signaling receptor binding activity. Involved in several processes, including animal organ development; open tracheal system development; and regulation of hemocyte differentiation. Located in several cellular components, including membrane raft; multivesicular body; and terminal bouton. Colocalizes with early endosome and late endosome. Is expressed in several structures, including ectoderm; ectoderm anlage; extended germ band embryo; gut section; and segment. Used to study obesity. Human ortholog(s) of this gene implicated in Mullerian aplasia and hyperandrogenism; osteogenesis imperfecta type 15; and osteoporosis. Orthologous to several human genes including WNT1 (WNT family member 1) (Alliance of Genome Resources, 2022).

RFLP (Restriction Fragment Length Polymorphism) is a technique that is widely used to detect variations at the DNA level. RFLP detection was carried out based on the possibility of differences in the length of the target DNA fragments produced after the cutting process with a restriction enzyme. Differences in the length of DNA fragments can be known after confirmation through electrophoresis, hybridization and visualization analysis. Application of the RFLP technique is used to detect genetic diversity, kinship, speciation and domestication, genome mapping, gene tagging, and construct cDNA libraries (Williams, 1989; Syamsurizal, 2019).

In silico test is a term for experiments or tests carried out using computer simulation methods. Usability test *in silico* is to predict hypotheses, give new discoveries or new advances in medicine and therapy. In silico can be used as a method to approximate real conditions into computer-based simulations using certain application programs or software. In silico testing is an initial study step before continuing with other tests such as in vitro and in vitro to help predict and provide hypotheses about the activity of a compound (Hardjono, 2013).

Based on the things that have been described, it can be seen that this study or research aims to analyze genetic variation in the WNT-1 gene sequence in*Formicidae* NCBI Popset 1871743971 using the RFLP technique (Restriction Fragment Length Polymorphism) with in *silico.*

Materials and Methods

Materials

In this study, the sequence used is the sequence of the WNT-1 gene in Formicidae NCBI Popset 1871743971 which will be downloaded in fasta format from NCBI. This sequence was sent by Lu, R. in his study entitled Habitat Filtering Shapes Ant Community Assemblies on Smaller Islands, But Competition on Large Islands (*https://www.ncbi.nlm.nih.gov/popset/1871743971 ?report=genbank*) This popset has 381 gene sequences, but only 30 gene sequences were tested.

Methods

Screening Candidate Restriction Enzyme

The restriction enzyme candidate screening process will be carried out using in silico tests using tools on the website http://insilico.ehu.es/restriction/. Later on this site will compare the restriction patterns of the many DNA sequences tested and will know the restriction enzyme that cuts it. The first step after entering the site is to select the "compare restriction pattern of many sequences" menu. Then upload the gene sequence in fasta format in the column provided. Then the alignment results will appear from each sequence, we can choose to discard the same sequence to simplify the analysis process. Next, select the menu "only restriction enzymes with known bases (no N,R,Y...)" this menu was selected in order to get a restriction enzyme with a definite restriction recognition side. And finally press the "get the list restriction enzyme" menu to get some restriction enzymes that will be used in the next stage.

RFLP

Restriction-Fragment Length Polymorphism (RFLP) was carried out manually *in silico* or virtual restrictions, this method uses the tools on the site https://www.benchling.com/ . o be able to access this site, you only need to register for an account using email and this site can be accessed for free. The first step is to enter 30 gene DNA sequences that have been downloaded from NCBI into the project folder on the Benchling site. After entering the site, click the scissors icon in the right corner of the site to do the cutting then a menu will appear and select the "find enzyme" tool, then select one of the predetermined restriction enzyme names, then select the "run digest" menu to perform restriction. And later the data will appear, as for obtaining an electrophoregram image can be done by pressing the "virtual digest" menu.

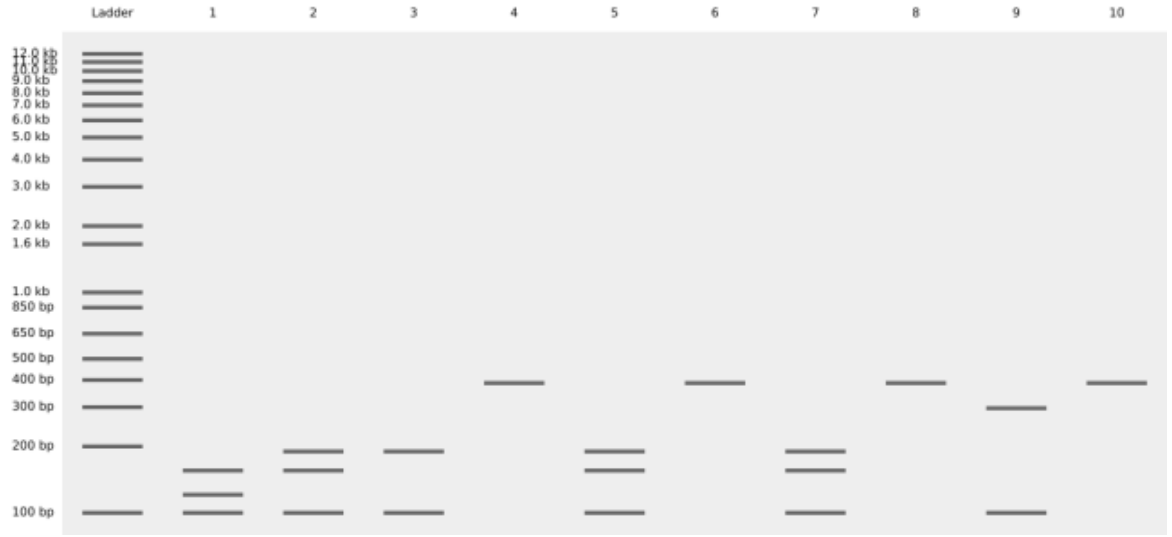
Results and Discussion

Screening Candidate Restriction Enzyme

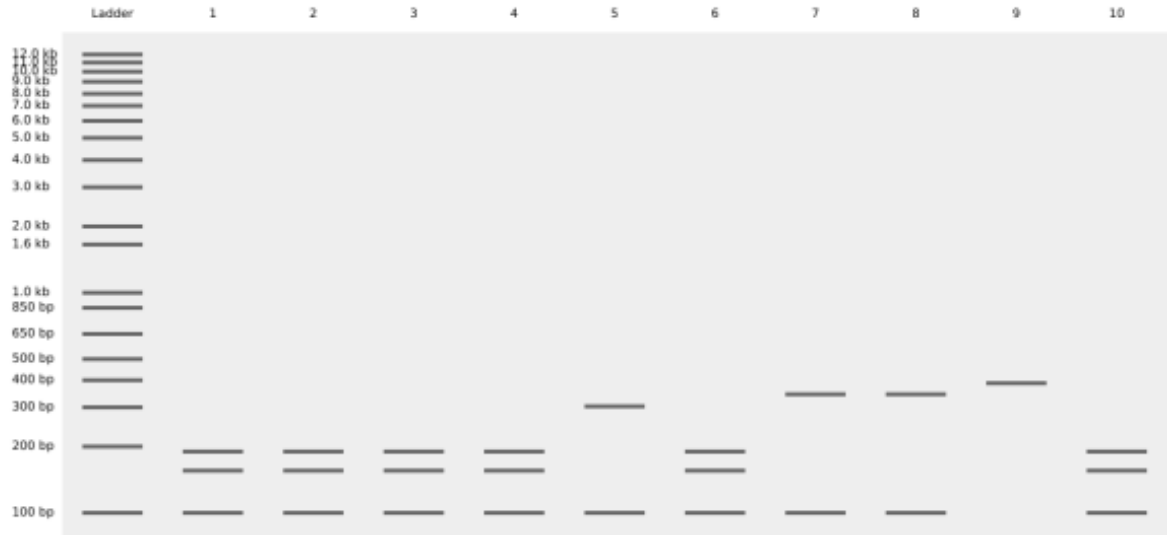
Based on the output obtained from screening restriction enzyme candidates on the website http://insilico.ehu.es/restriction/ , there are 381 restriction enzyme recognition sides, one of which is GA^TC which is recognized by the DpnI enzyme. This recognition side was chosen because it shows variations in the cutting side of the WNT-1 gene sequence in Formicidae in PopSet 1871743971.

RFLP

Picture 1 . Electrophoregram of restriction result with DpnI enzyme with in silico. Left: Ladder Life 1 kb Plus, (1) MN159810.1, (2) MN159811.1, (3) MN159812.1, (4) MN159813.1, (5) MN159814.1, (6) MN159815.1, (7) MN159816.1, (8) MN159817.1, (9) MN159818.1, (10) MN159819.1



Picture 2 . Electrophoregram of restriction result with DpnI enzyme with in silico. Left: Ladder Life 1 kb Plus, (1) MN159820.1, (2) MN159821.1, (3) MN159822.1, (4) MN159823.1, (5) MN159824.1, (6) MN159825.1, (7) MN159826.1, (8) MN159827.1, (9) MN159828.1, (10) MN159829.1



Picture 3 . Electrophoregram of restriction result with DpnI enzyme with in silico. Left: Ladder Life 1 kb Plus, (1) MN159830.1, (2) MN159831.1, (3) MN159832.1, (4) MN159833.1, (5) MN159834.1, (6) MN159835.1, (7) MN159836.1, (8) MN159837.1, (9) MN159838.1, (10) MN159839.1

Table 1. Allel Frequency of WNT-1 Gene in Formicidae based on In Silico RFLP Results

Restriction Enzyme	Restriction Recognition Site	Cutting as Much	Number of Fragments present (N=30)	Percentage of Fragment Presence (%)	Allel Frequency
*Dpn*I	GA^TC	0	6	20 %	0.2
		1	8	27 %	0,267
		2	13	43 %	0,43
		3	2	6,7 %	0,067
		4	1	3,3%	0,033

From the table, we can know that of the 30 DNA gene sequences cut using the restriction enzyme DpnI restriction recognition site GA^TC produced 5 types of cuts, some of which the enzyme did not cut at all, cut 1 time, cut 2 times, cut 3 times, and cut 5 times. Where the fragment length of the cutting results varies from the shortest 26 bp and the longest 303 bp.

Restriction enzymes that do not cut at all have the presence of fragments as much as 20%, restriction enzymes that cut once have the presence of fragments as much as 27%, restriction enzymes that cut twice have the presence of fragments as much as 43%, restriction enzymes that cut 3 times have the presence of fragments as much as 6.7% and restriction enzymes that cut 4 times have the presence of fragments as much as 3.3%. With the highest percentage of presence, the restriction enzyme that cuts 2 times appears on 13 DNA fragments, and the lowest percentage of presence, the restriction enzyme that cuts 4 times, appears on only 1 DNA fragment.

Conclusion

Based on the experimental results and in silico RFLP analysis, we can conclude that there are genetic variations from a total of 30 WNT-1 gene sequences in Formicidae found on NCBI in PopSet 1871743971. This is due to the emergence of 4 kinds of cutting alleles with varying fragment lengths for each allele.

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